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REGENERATION IN PLANTS. II.[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXIX.

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(WITH NINE FIGURES)

WOUND STIMULUS.

THE influence of the wound has been put forward many times, either as the direct cause, or as an important factor in regeneration. WIESNER (19) has suggested that between the wounded part and the new structure formed there is a direct causal connection, due to substances developing in the wounded cells and passing to other parts, there inciting the reversion of mature cells into the meristematic condition. GOEBEL (4, p. 204) also believes the wound stimulus to be a factor. KLEBS (9), on the other hand, rather discredits this idea and thinks that the wound in itself is of no importance in regeneration. The possibility of the wound having a far reaching influence is not at all improbable, for many well-known cases of traumatropism show how cells may be affected at a distance from those actually concerned in the wound. Of the many experiments to determine if such an influence is operating, only a few need be mentioned, and in these cases only the results will be given.

It was seen that with Phaseolus the removal of the stem above the basal primordia is followed by the development of the latter. The removal of the cotyledons, while causing wounds still closer to the young bud, produces no such development; nor does the severing of the stem as close as possible below the primordia. Wounding the stem by cutting notches immediately above the primordia, as

[1] The first paper was published in BOTANICAL GAZETTE 40:97–120. 1905.

deep as four-fifths through the stem, has no influence. String was tied tightly around young stems, just above the primordia, and as the stems grew the string cut in deeply on all sides; no results followed. Longitudinal slices, the length of the epicotyl and three-fourths through the stem, produced no results.

Experiment 38.—Notches cut at different points in a spiral around the stem, so that all the bundles were severed, failed to incite the buds below.

Experiment 39.—Five plants 70–100^{cm} high, 6–9 internodes long, and with lower internodes old and hard were used. The tip of each plant was cut off and also the buds from all the nodes below. In three plants the basal primordia produced shoots; one plant died; and the others remained alive, but no shoots formed. Here the wound effect, if there was such, traveled through a distance of nine internodes. A wound, however severe, seems unable to cause the buds to develop if it does not include the complete removal of the growing apex; and even on a large plant the removal of the very tip is all that is necessary. Here, as in the willow and other plants (see experiment 43), the effect of the wound passes only down the stem; a wounding or a complete severance at any point along the stem has no effect on the buds above this point. As will be brought out later, the opposite is true of the roots; that is, the influence of the removal in inciting new roots only passes upwards. This would hardly be true if it were due to the diffusion of substances formed in the wounded cells, as WIESNER supposes. GOEBEL (5) has found that in Bryophyllum no wounding at all is needed to produce shoots on the leaves. He encased all the buds on the shoot in plaster so as to prevent further growth, and after a time the buds on the leaves developed. If the leaf blade of Cyclamen is cut off new leafy structures arise along the margins of the petals. But WINKLER (19) has shown that this removal is not necessary. He left the blades intact, but incased them in plaster, and soon the leaf-like outgrowths appeared as when the blade was removed. The blade was not injured or wounded in any way by this treatment, though undoubtedly some of its activities were suppressed.

As will be described in experiment 40, I inhibited the growth of the apex of Phaseolus by placing it in a hydrogen atmosphere. The basal primordia in the air below promptly developed. When the hydrogen was removed, the apex continued growing. Placing the roots in plaster, which inhibited any further growth on their part, also resulted in the development of roots along the stem. In these cases there was no wounding anywhere. It appears that while regeneration follows the removal of certain parts, neither this removal nor the wound incident to it are necessary, since the regeneration occurs equally well when the part is left uninjured and certain of its activities suppressed. The wound, therefore, is not in itself any part of the stimulus.

CORRELATION.

By correlation is meant the influence which one organ or part may exert over another. That the removal of certain parts leads to changes within the plant that may modify markedly the growth or function of other structures is a matter of common observation. Examples of this interdependence among the different members of the plant body are abundant. JOST (**8**) has shown that in Phaseolus the mere presence of the leaf is a necessary condition to the development of the bundles of the leaf trace. PISCHINGER (**16**) determined that if the large cotyledon of Streptocarpus be removed the small functionless one will develop into a large one. According to GOEBEL (**6**, p. 809) the early removal of foliage leaves induces the bud scales to develop into the foliar structures. If the upper end of a Taraxacum root be cut away, removing all the buds, new buds soon arise from the cortex below; this is true of many roots. IRMISCH and others (**10**) have shown in many species that if seedlings be cut off below the first node, new buds arise out of the tissue of the hypocotyl. The removal of the growing tip on many shoots is followed by the development of the dormant axillary buds. As is commonly observed in cultivation, if the lateral roots be destroyed, their place is taken by new roots, which otherwise would not have developed. GOEBEL (**2** and **5**) showed that in Begonia and Bryophyllum, and this is probably true for many other plants, the removal of all the buds on the shoot will result in the development of buds on the leaves.

The process by which such an influence is exerted by one part over another is the main problem to be solved in regeneration. GOEBEL attempts to explain it in one of two ways: either (1) the one part monopolizes the nutritive material to such an extent that the other parts concerned cannot obtain sufficient to enable growth to go on; or (2) he applies SACH'S *Stoff-form* hypothesis. According to this (**17**), there are formed in the plant small quantities of different substances, presumably of enzyme-like nature, each one having the capacity to incite the formation of a definite structure. These substances are supposed to move in definite directions, and where they accumulate in sufficient quantities start the development of the particular structure they are concerned with. In GOEBEL'S opinion (**4**, p. 204) the influence of external conditions are of little account in regeneration, the important cause being "the direction in which the constructive material moves." GOEBEL says (**3**, p. 42) "the vegetative points act as centers of attraction for the plastic material, their influence being stronger or weaker according to their position." In Bryophyllum, for example, the apex of the shoot is the strongest, then the lateral buds, and last of all the vegetative points on the leaves; so that the apex is able to draw to itself the greater part of the "constructive material;" but if this apex is removed, the lateral buds will be able to "attract" this substance; and in the absence of these lateral buds, the growing points on the leaf are able to appropriate it. In Begonia no growing points are present on the leaf, but when it is removed GOEBEL says bud-forming material accumulates at the base and induces the formation of buds there. If this material, formed in the leaves, moves toward the base of the leaf and passes out because it is "attracted" by the growing points on the stem, just why it should continue to flow in that direction and accumulate at the base, when all connection with these "centers of attraction" is broken, is one of the unexplained difficulties that beset this hypothesis on every hand. MORGAN (**12**) has strongly objected to this theory, but his evidence against it does not seem to me to be necessarily fatal. GOEBEL in a recent paper (**5**) is inclined to lay less stress on it than formerly, asserting that the non-development of the buds on the leaves is due to a checking influence exerted by the buds of the shoot; "but," he adds, "whether we are here dealing with a stimulus transmitted along the conducting system, or whether the building material (*Baustoffe*) flowing in the conducting channels is attracted more strongly by the shoot vegetative points than by those on the leaves remains uncertain."

DRIESCH (1), who has worked on animals more than on plants, recognizes in correlation the dominant factor in regeneration, and claims that the absence (*Nichtmehrvorhandensein*) of the one part is the cause of the development of the other. The plant, according to him, is not influenced in regeneration by external factors, but being sensitive to something lacking endeavors to replace it. Somewhat analogous to this is NOLL'S idea (14) of a body-forming stimulus (*Körperformreizen*), by which he implies that there is an innate impulse in the organism toward a definite form, and when a part is removed the resulting disturbance (*Formstörung*) acts as a stimulus to the reconstruction of the whole.

Such hypotheses as these are at present as incapable of demonstration as they are of refutation, and can only serve a useful purpose if they form the starting point for experimentation. Unfortunately they can scarcely be said to do that. Quite different from these is the view held by KLEBS (9, p. 109), who believes that the removal only serves to bring about those conditions, such as accumulation of moisture, changes of a nutritive nature, etc., which would under all other circumstances cause a similar development. To take a specific case, the normal absence of roots on the stems of Salix, KLEBS says, is due to the retarding influence of light, of dry air, and to the fact that the water is being used by the leaves and young parts. When these conditions are supplanted by those of moist air and abundance of water, the roots develop quite independently of any removal or wounding. The experimental evidence that follows shows, however, that the problem is much more complicated than this.

The experiments described have shown the dependent relation that exists between the growth of the apex and the non-development of the buds below. On Phaseolus the basal primordia do not develop so long as there are buds above them developing. Indeed, only one bud is sufficient for this; for if the upper part be cut away and all the buds but one be removed, the basal buds do not start.

Experiment 40.—The stems were cut off at the second internode, and the buds from one side of the base of this were removed; and in some cases not only the bud but the leaf and one-half the diameter of the epicotyl for its entire length was sliced away. The remaining bud grew vigorously, but neither of the buds at the base developed. If at any time this bud was cut away those at the base promptly started.

Experiment 41.—The buds at all the nodes were removed from a larger number of plants, leaving the growing tip and the basal primordia; the latter did not develop. When the tip also was removed they started promptly, even though they were separated from it by a distance of 70^{cm}—six to nine internodes. *Figure 1* shows one with three internodes.

FIG. 1.—*Phaseolus:* Tip and all upper buds removed; buds at base soon developed shoots.

It has been shown that no amount of wounding short of the complete severance of the stem will produce this result. GOEBEL (2, p. 386) says that in Circaea if the central orthotropous shoot be allowed to grow in a dark chamber, it has the same effect on the lateral plagiotropic shoots as if it were removed; that is, one or more of these become orthotropous.

Experiment 42.—Young plants of Phaseolus were taken when the epicotyl was 5 or 6^{cm} long, the first pair of leaves just unfolding and the apex with the leaves directed into a dark chamber. The lower part of the stems were in light and the roots in soil. The parts in the dark elongated rapidly and soon were completely etiolated, but in no case did the buds below develop. Similar experiments with Salix showed this would not cause the axial buds below the part in the dark to develop. It would look as though a complete removal of the apex is necessary to start into activity the latent growing points below, but experiment 43 shows that this is not so, for there the tips of the four young plants were passed into a bell jar and sealed air tight with wax, and through the bell jar a continuous current of hydrogen gas was passed. The growth slowed down, and after about twenty-four hours ceased entirely; and in a few days the buds in the axils of the cotyledons below started to develop and grew quite vigorously. Upon the removal of the bell jar the apices of the shoots continued to grow. This shows that only a cessation of certain activities, presumably those concerned with growth, is necessary; and it has been shown above that however this may act on the buds below, it is not through any disturbance created in nutritive or water relations.

GOEBEL (5) has shown that if in Bryophyllum all the buds on the stem are prevented from growing by encasing them in plaster, the growing points on the leaves develop. HERING (7) showed that the small cotyledon of Streptocarpus would develop both structurally and functionally into the large one, if the latter were prevented mechanically—by plaster—from growing. WINKLER (19) has found that by a similar treatment of the leaf blade of Cyclamen the new leaf-like structures that develop when the blade is removed will arise from the margin of the petiole. He leaves us in doubt as to whether he selected leaves that had entirely ceased growth before his experiments began. He thinks that the regeneration is due to the interruption of one or more of the functions; either respiration, transpiration, or photosynthesis. My results show that for Phaseolus and Salix cessation of neither transpiration nor photosynthesis will cause regeneration. Respiration is checked in the hydrogen, but what other changes may be involved it is impossible to say.

On those plants whose growing tip soon ceases activity and dies, as *Syringa vulgaris*, I have not been able to induce the axillary buds to develop by removing the apical part of the shoot. In all of many cases tried, however, in which the terminal growing points continue their activity during the growing period, their early removal was followed by the sprouting of latent buds below (*fig. 2*). This is true even in those plants whose annual shoots are without branches, and in the axils of whose leaves the buds cannot be seen even with the aid of a lens.

Lack of space prohibits a detailed description of these experiments, and only the results need be given. In the majority of cases it is the buds near the apex that start (*fig. 3*), but occasionally almost every one on the shoot starts; and in one case, on a shoot of Salix, only those at the base started. The young shoots were either cut off and the base placed in water, or else they were left attached to the plant. If the tip is cut off and also all the leaves, the buds develop. Or if the tip is removed and the shoot placed in the moist chambers mentioned above, so that transpiration is entirely prevented, they also develop. In these moist chambers, however, with the tips still intact, no development of the buds occurs. If the shoot is removed and placed in darkness for a few days, until the food is mostly exhausted by the rapid growth, and then the apex cut off, the lateral buds still develop. The removal of the leaves has no influence on the buds, for from many shoots not only were all the larger leaves carefully cut away, but even those

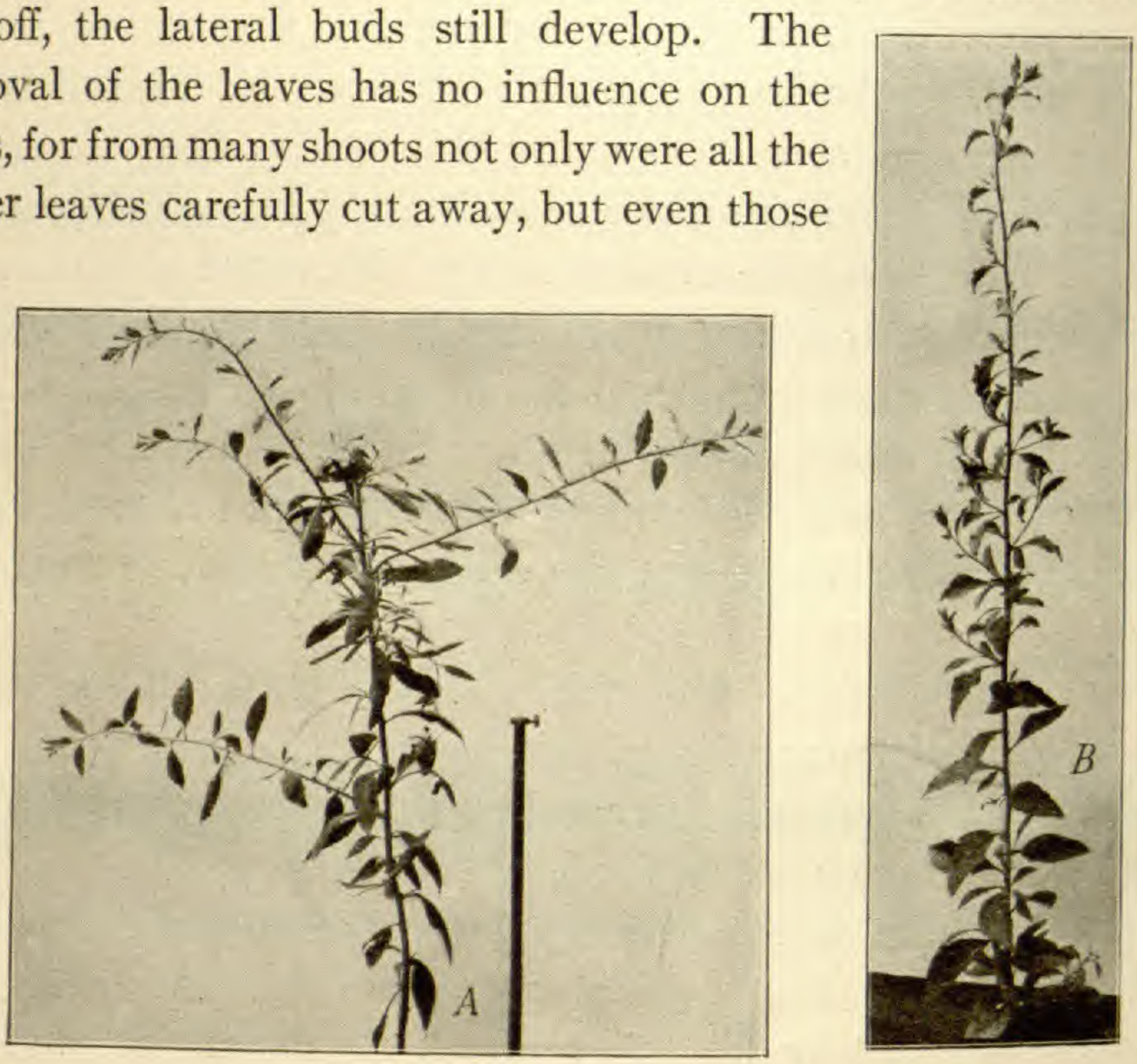

FIG. 2.—*Lycium halimifolium.* Two similar shoots were selected and from *A* the apex was removed; the photographs show them both three weeks later.

still folded in the buds, and in no case did the growing points develop. But when in addition the tip of the shoot was removed, they at once started. While these experiments were mostly on Salix, other plants, such as Cornus, Lycium, Ficus, Oleander, etc., gave similar results. Here, as in the basal primordia of Phaseolus, no matter how vigorous and well-nourished the plant may be, or how abundant its water supply, with the growing apex of the shoot intact, the young axillary buds remained dormant. When this tip is removed, the bud starts to develop even with a loss of water or in a starved condition. This capacity is greatest in the young shoot, and gradually declines with age as the seasonal growth ceases. In annuals, like Helianthus, whose axillary buds finally develop branches, the removal of the apex from young plants causes the buds to develop at once. *Silphium integrifolium* has a leafy stem, unbranched until late in the season when the flower branches arise; but if the apex is early removed, the minute axillary buds promptly produce branches.

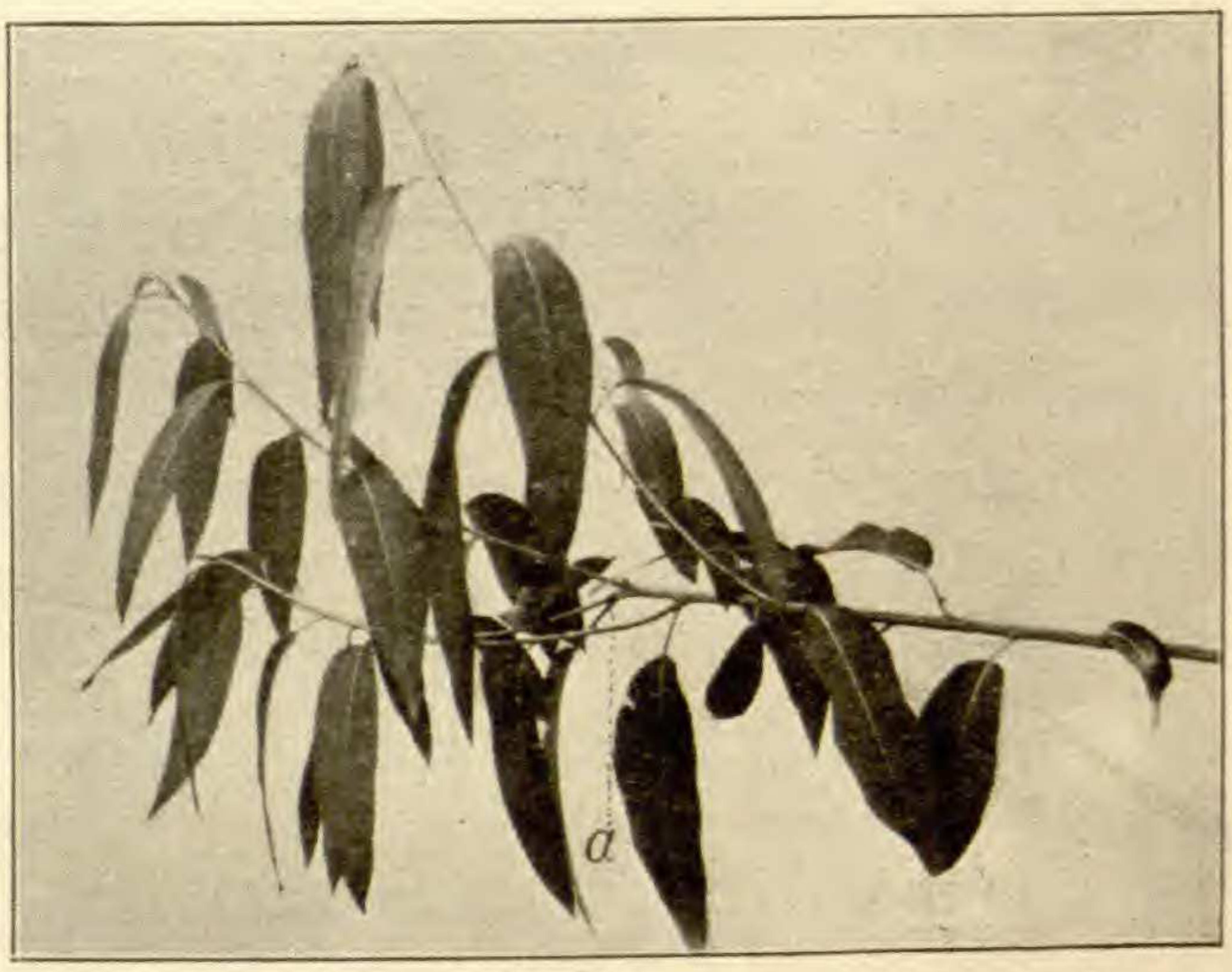

FIG. 3.—Young shoot of *Salix amygdaloides*. The apex was cut off at *a* and four branches developed below this.

Not only does the growing apex exert an influence felt by the growing points below it, but those along the shoot exert a similar influence upon those lower down. VÖCHTING (18) showed that in isolated pieces of Salix stem only the buds toward the upper end of the piece develop. PFEFFER (15) found that if the upper buds are placed in plaster those lower down start. As a series of experiments on polarity will be published in an article to follow this one, only brief mention will be made here of the experiments in this connection. The plant used was Salix.

Experiment 43.—Four pieces of two-year old stem, 35^{cm} long, were placed with the basal ends in 6^{cm} of water. Two were erect, with the remaining parts in the moist air; the upper 8^{cm} of the other two were in a bell jar through which a current of hydrogen passed. In a few days the buds at the upper part of the first two started to develop; and, so far as could be observed, simultaneously with them those on the other two pieces just below the part in hydrogen. Those in hydrogen were not killed, and when the gas was removed started to grow. In a few weeks their shoots had surpassed those below them, which had now almost ceased growing.

Experiment 44.—Twelve similar pieces of stem were selected, 30^{cm} long. On three the buds were left only on the upper third;

on three more only on the middle third; on three more only on the lower third; while all the buds were left on the last three. These were all placed horizontal in moist air. On the last three mentioned only the upper buds formed shoots; and at the same time most of the buds, especially the uppermost ones, started on all the other pieces. Many tests showed that any bud at any place along the stem would develop if the buds above it were prevented from doing so. Here again, it is not a question of nutrition or water, for the stems and buds are filled with reserve food, and in a constant spray (in which the experiment was repeated) there can be no lack of water.

Whatever the influence of the growing buds may be, it is felt only on those below them, and not on the buds above them (that is, toward the apex of the axis). In the experiment just mentioned, in which the upper buds were inhibited by hydrogen, those below had gotten a good start and were forming shoots, but when the hydrogen was removed, the upper ones developed as usual.

Experiment 45.—A piece of Salix stem, 30^{cm} long, was placed so that the 12^{cm} in the center was in a continuous spray, and the two ends in quite dry air. The buds in the central portion swelled up and burst open before the others showed any signs of swelling. The whole piece was then placed horizontal in moist air. The buds at the apical end soon enlarged and developed shoots; but those at the lower part did not. These central buds, while able to prevent the buds below them from developing, had no influence on those above them. Finally, all the young shoots and buds were cut off from the upper two-thirds of the piece, and the upper ones on the basal third promptly started.

If the entire piece is surrounded by the same conditions, the shoots all appear at the apical end; but by placing the basal end in water and the rest in dry air, the buds in the water or close to the surface start first; but soon the upper ones commence to grow, apparently indifferent to those below them; and as the young shoots increase in size, those below become less vigorous and are often finally suppressed. Other experiments of a kindred nature might be mentioned, but these will suffice to indicate that the development of the buds at any region along the stem tends to suppress those below them from developing, but does not influence those above them.

Here we are dealing with growing points already laid down, but the same principle holds where these do not exist. If we place a root of Taraxacum in a moist condition, the buds at the top will soon develop; but if we remove all these buds, entirely new ones will be organized and develop. If these be prevented from growing, lower down others will be organized, as GOEBEL has shown (**2**, p. 492). Here the organization of new shoot primordia along the root does not occur so long as those at the top are allowed to grow. Here again we cannot attribute this to the monopolizing of the food or water by the upper part, for these are abundant everywhere. There is a direct relation between the growth of the shoots at the top and the non-formation of buds lower down, entirely independent of these two factors.

The development of new roots when those present are removed shows a similar phenomenon. The behavior of Phaseolus in this connection may be briefly mentioned. Plants grown with roots in water cultures developed a vigorous root system. From some of these all the lateral roots were removed, leaving only the main root. Soon numerous new lateral roots arose and grew vigorously; these were cut off and still others came on, though not so vigorously. In the mean time no new lateral roots had come out among the older ones on those that had not been so treated. If we cut off the main root transversely, numerous roots arise just above the cut; and if we cut away the whole root system by severing the stem at the base, new roots arise on the lower part of the stem. Sometimes the roots are so numerous here that I have counted eighty-one coming out of the lowest centimeter of a Phaseolus stem less than 5^{mm} in diameter. It was found almost impossible to produce roots on any part of the stem that is in direct connection with roots below, but when this connection is broken roots promptly start.

Experiment 46.—Local regions of several stems were surrounded by water in glass cylinders, as in *fig. 4*. On some the roots were intact, and from others the stem was cut off at the base. The roots and the lower ends of the stems were in water. On those with roots still attached no roots formed on the part of the stem surrounded by water; but the others produced abundant roots there and also at the lower end (*fig. 5*). Finally, from one of the former the root system was removed by severing the stem at the base, and vigorous roots then appeared in the cylinder, as well as on the lower end of the stem.

The influence exerted by the roots very evidently passes along the vascular bundles, for the transverse cutting of these at any place leads to the origin of roots just above the cut. The cutting of the cortex as deep as the bundles has no results. If a notch be cut in the stem so as to sever some of the bundles, roots appear only above, not below the notch (*fig. 6*). If the stem is cut off near the base, roots come at this place; but if it be cut off further up, the roots come there even more vigorously. If a series of notches, either one

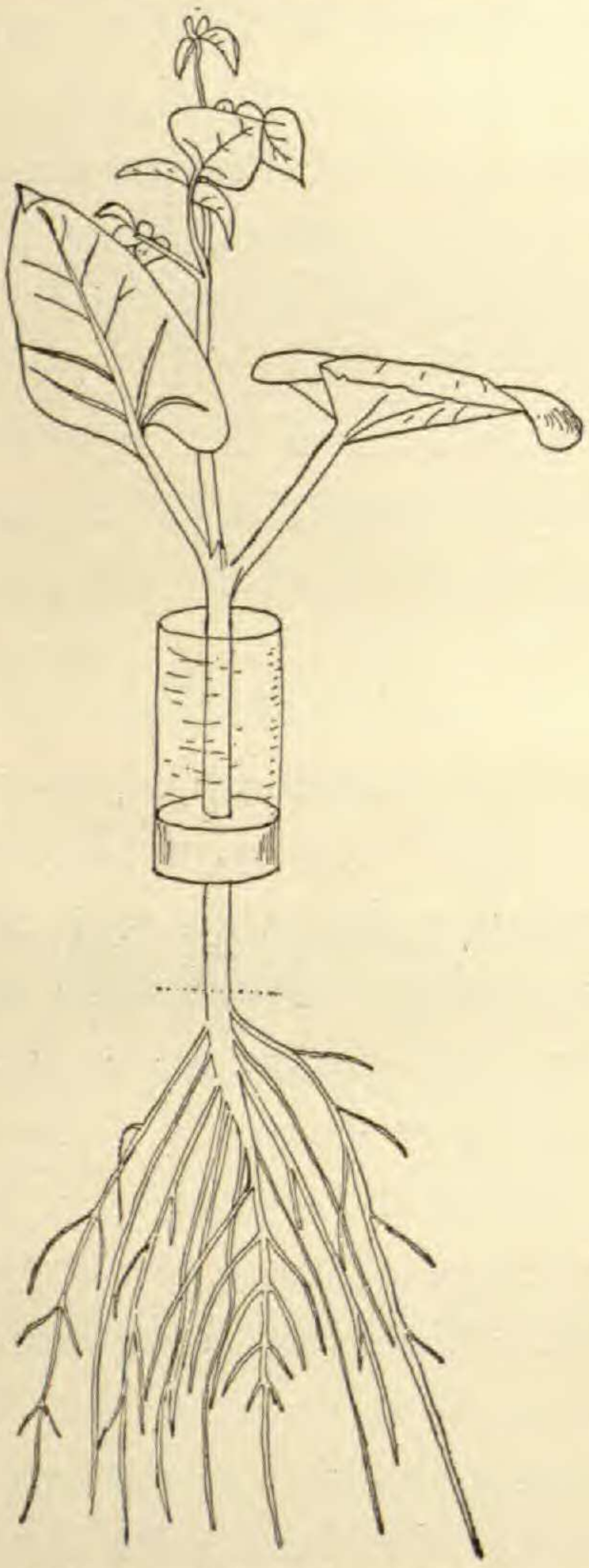

FIG. 4.—*Phaseolus:*—A short glass tube attached around stem and filled with water. Roots, grown in water culture, left uninjured and submerged. No roots formed on stem within tube.

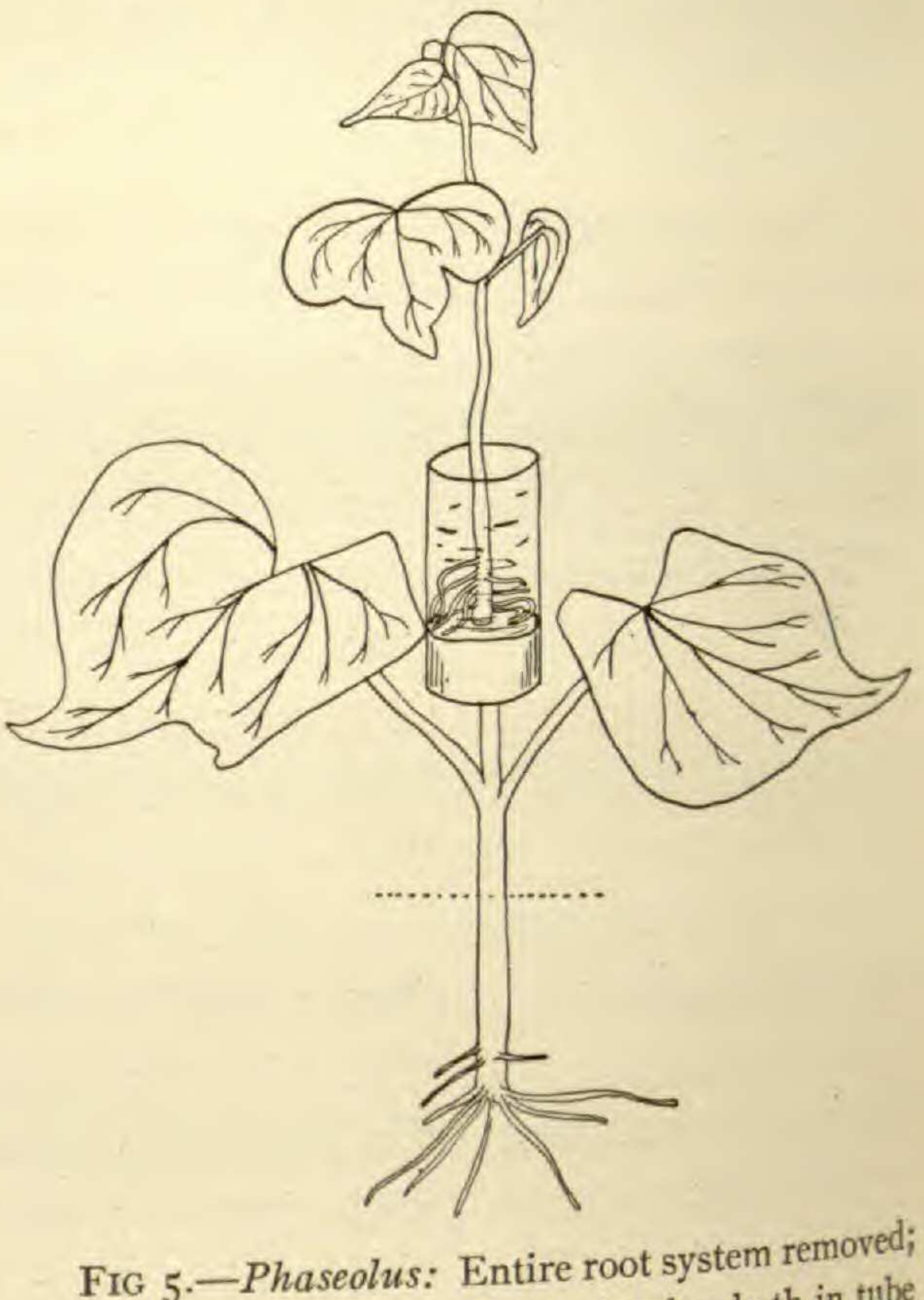

FIG 5.—*Phaseolus:* Entire root system removed; otherwise as in *fig. 4*. Roots develop both in tube and in water at base of stem.

directly above the other or on different sides, be cut, roots come from above each of them, but more vigorously from the upper ones, due probably to that part being nearer the source of food supply. Or when stems are cut through, some at the base and some higher up, the roots appear perceptibly sooner on the latter. No matter where the stem is cut off, roots develop immediately above this point, showing that the pericycle has the power to produce roots at any point. Yet, as has been stated, if the stem be cut off, say near the base, roots come only here, though the whole stem is submerged in water. But when the cut ends of the stems were encased in plaster so as to prevent roots from coming there, they came further up. Also stems whose lower internodes were 10–12^{cm} long were placed in water, and every day 0.5^{cm} was cut off. In ten days roots appeared scattered along the remaining part.

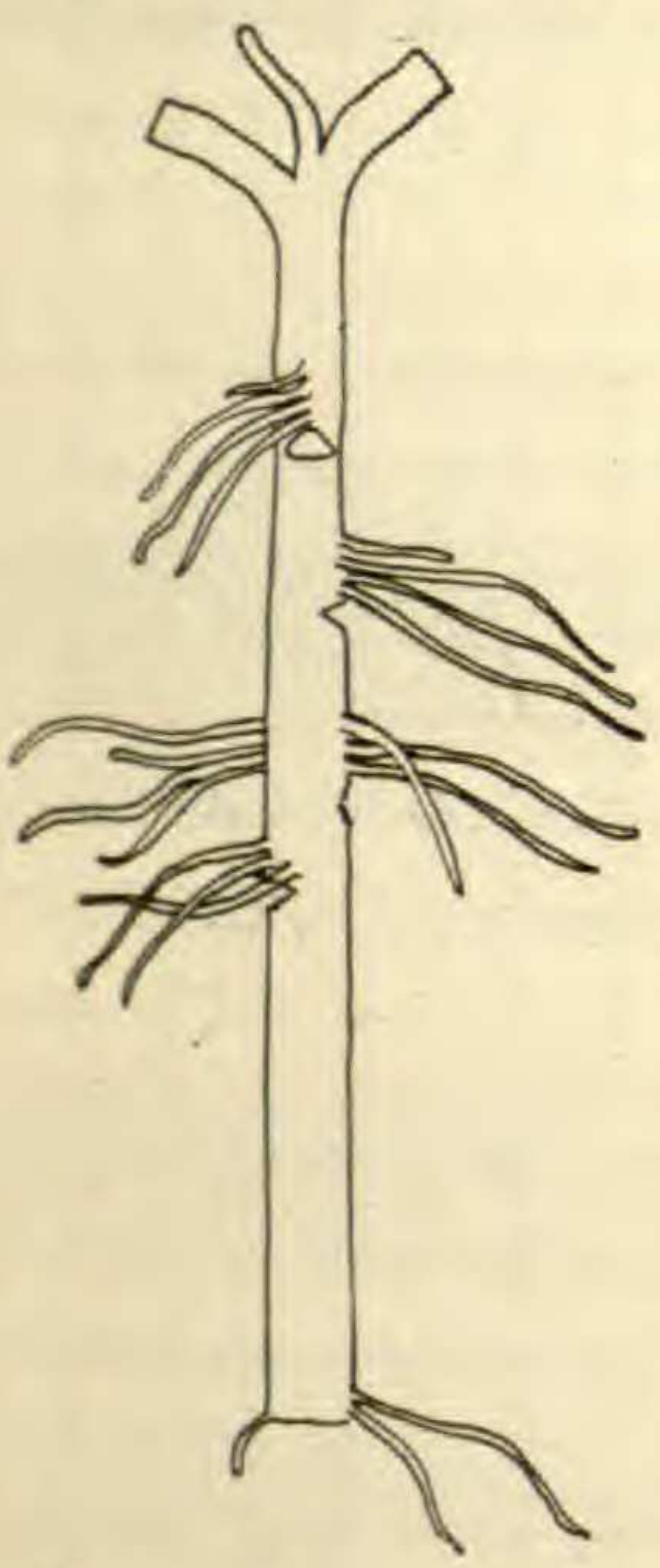

FIG. 6.—*Phaseolus:* Lower internode of stem notched on different sides and completely submerged. Roots appear only above the notches.

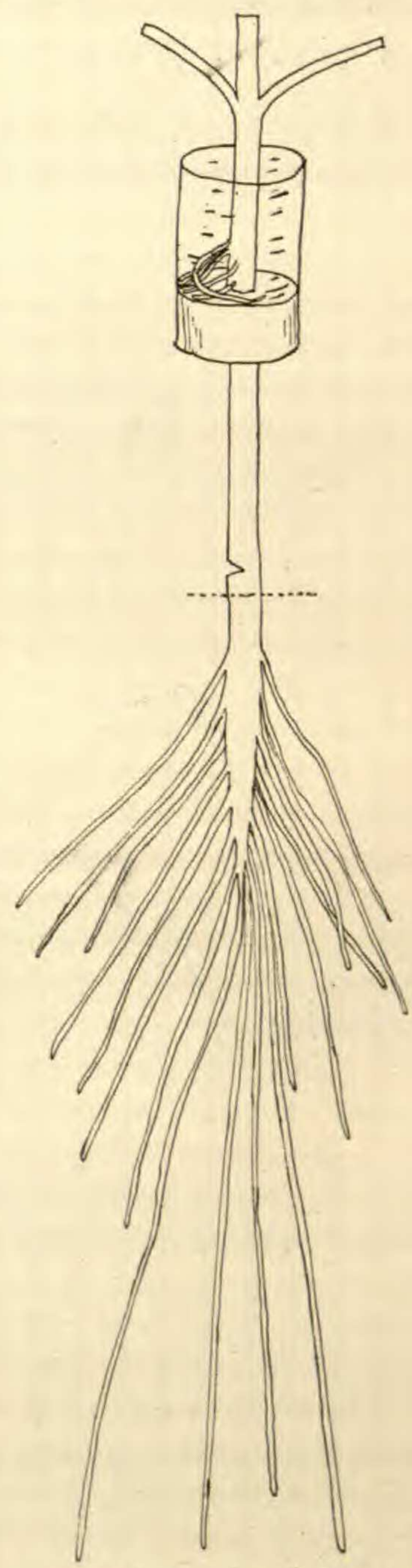

FIG. 7.—*Phaseolus:* Part of stem surrounded by water as in *fig. 4*. Roots intact and submerged to dotted line above which stem is notched to center. Roots appeared in tube, arising only on side directly above notch.

Experiment 47.—Portions of stems with roots intact were surrounded by glass cylinders 4–5^{cm} long and made air-tight at each end by means of rubber stoppers and wax, and opening into a vessel containing a 3 per cent. solution of ether. This seemed to anæsthetize the stem without killing it, and roots appeared just above this portion. The effect of the anæsthetic probably was to prevent any passage of stimuli through this part of the stem either up or down. Experiment 30 is instructive in this connection. It will be recalled that surrounding the stem at any place with water in glass cylinders will not start roots at that place if the connection with the roots below is unbroken; but, as shown in *fig. 7*, if a notch is cut in the stem some distance below, thus severing connection with the roots, roots will appear above the notch in the water, apparently coming from those bundles severed by the notch. In other words, the water supplies a favorable condition for root development, and the cells are able to act as soon as the connection with the roots below is broken. If the air be moist, roots come out also immediately above the notch.

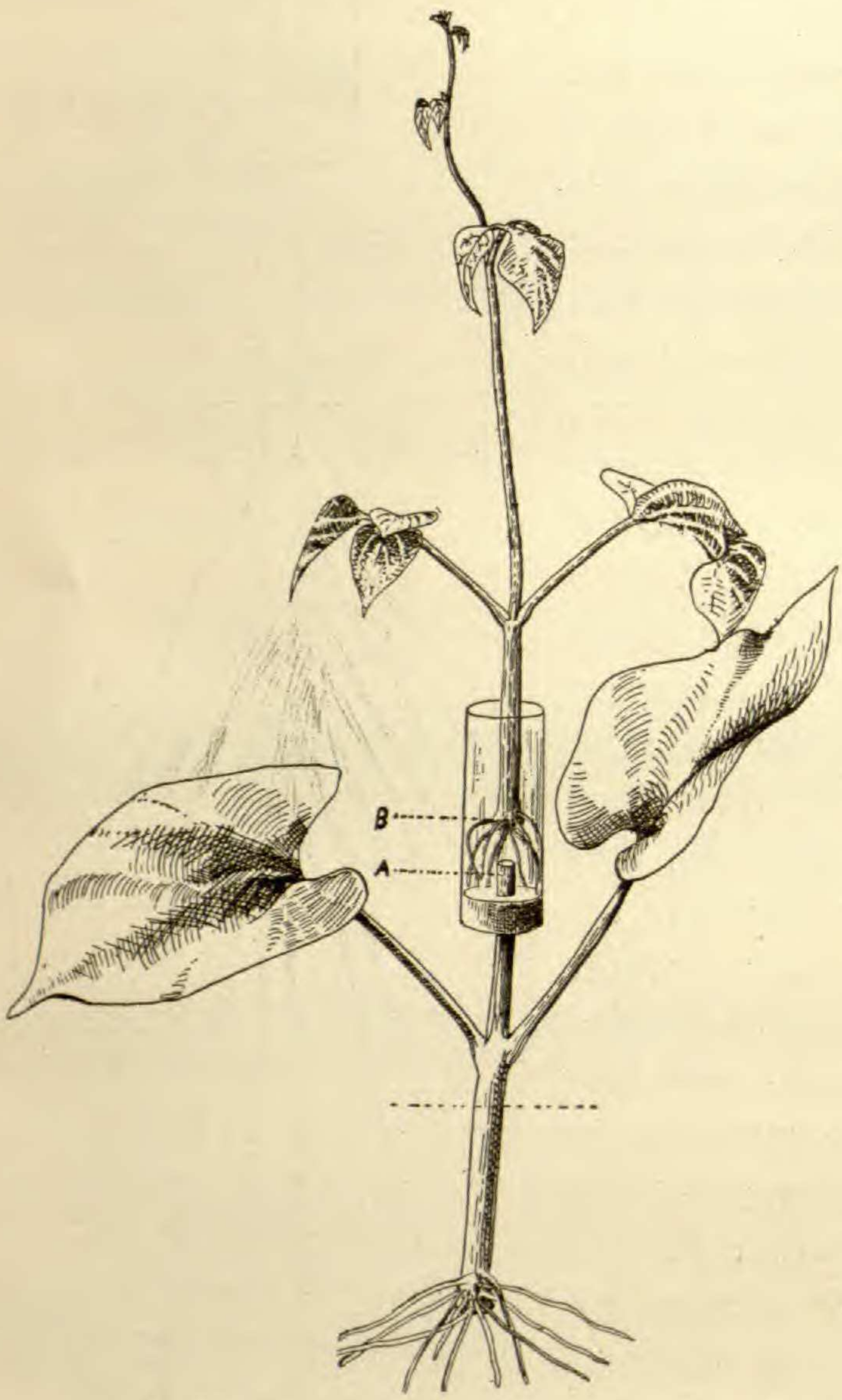

FIG. 8.—*Phaseolus:* Stem cut off at base; lower end submerged. Portion of next internode above surrounded by water as in *fig. 4*, and stem severed; *A*, *B*, ends slightly separated. Roots appeared only at *B*.

When a stem is arranged as in *fig. 8*, roots always arise on the part just above the cut, never on the part just below it, even though this piece is inverted with its end in water, as in *fig. 9*. This difference in the behavior of the cells at the two places cannot be due to any difference in the amount of water or nutritive material available at the two places, for both may be saturated with water and equally well provided with food. Nor can it be that the cells just below the cut have any less capacity for root production than those just above; for if the cut be made a little lower down, so that the cells which formed the upper part of the lower piece now form the lower part of the upper piece, they promptly produce roots. It is evident that there is some factor operating on the cells at the one point that is not present at the other; and it seems equally evident that this is not a condition of moisture, of nutrition, or of a wound influence, for all of these are equal in each case. As stated above, when no cut is made there is no tendency for any of these cells to form roots; but as soon as an incision is made, there is a change in the behavior of the cells above it, while those below remain uninfluenced. In the present state of our knowledge there seem only two possible lines of explanation: (1) as a result of the incision, new conditions are added to the cells above the cut which serve as a stimulus inciting them to root production, conditions not acting on the cells just below the cut; or (2) the cells above the cut have been relieved of some influence that previously prevented their growth, an influence still acting on the cells below the incision, and which acts on all the cells when no incision is made.

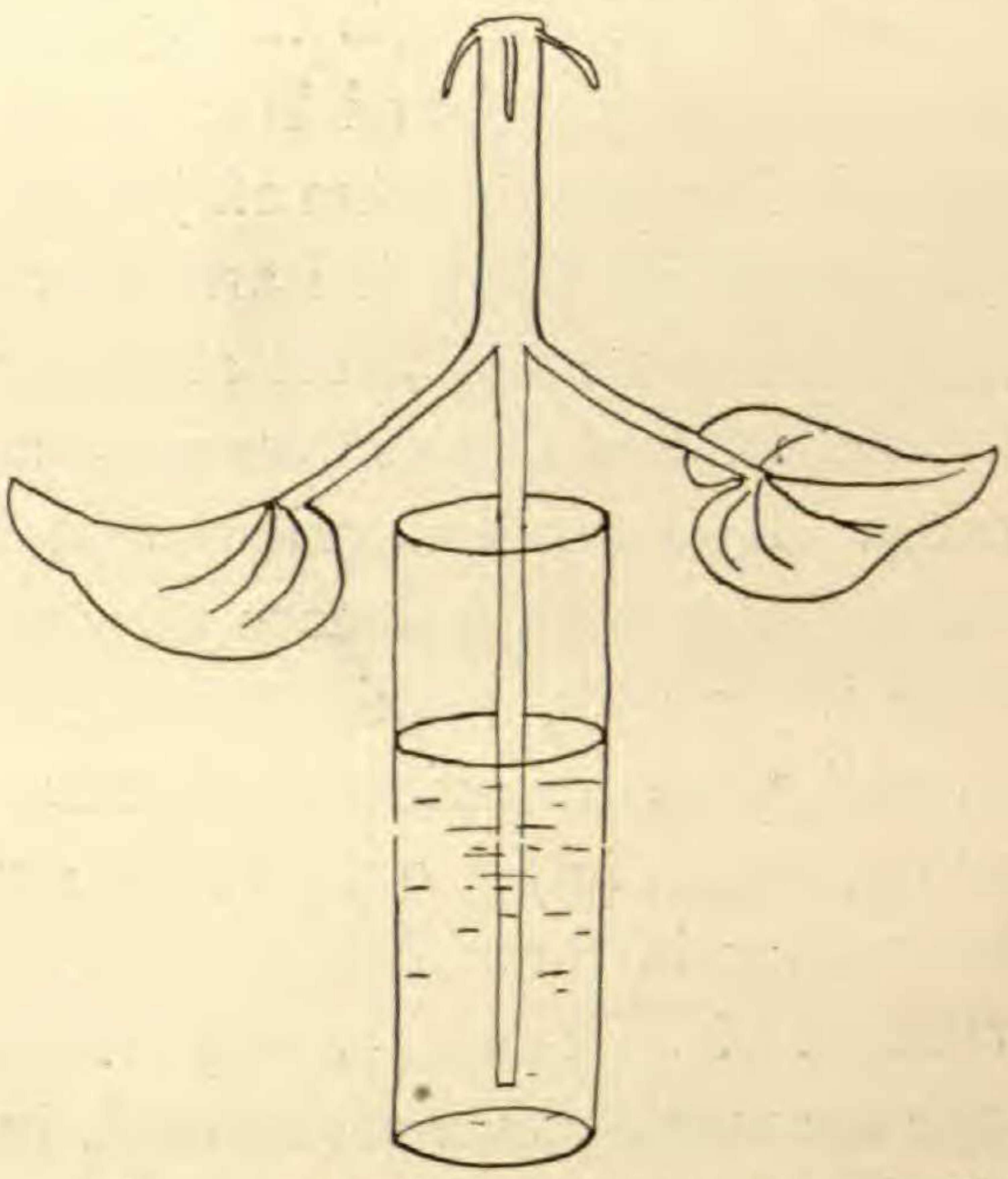

FIG. 9.—*Phaseolus:* Inverted stem with first pair of leaves and adjacent parts of internodes. Apex in water, base in moist air. Roots arising only on base.

Recurring to the first idea for a moment, we have noticed that there are two different conceptions current. The one is that these new conditions added are the results of changes induced by such factors as nutrition, moisture, wounding, light, gravity, aeration, etc. While light retards the development of roots in this case, it does not modify their distribution; and as the roots occur in the same way whether the piece is erect, horizontal, or inverted, gravity cannot be a determining factor. The other conception is that of specific formative substances, which in this case, moving toward the base, would accumulate at the lowest part and incite the formation of roots there. As this theory is to be discussed later, only two cases will be mentioned here to show its inefficiency. If in Salix, instead of cutting the stem off just above a bud, the bud is cut entirely away from the stem, it starts to develop just the same; or, as in *fig. 7,* where a notch is cut at the base of the stem, there can be no accumulation of these substances further up where the roots actually occur. It is not impossible that the upper parts, especially the leaves, exert some influence on the formation of roots on the stem. If so, it is not through the transpiration current, for the roots develop as well when transpiration is entirely checked as when it is quite active. Also, in *fig. 9* the leaves are transpiring and a current must be passing in through the end that does not produce roots. The removal of all leaves greatly decreases the vigor of the roots and also the number that are formed, but the same result is obtained by placing the leafy part in the dark, or in an atmosphere free from carbon dioxid. The fact that pieces of the internode produce roots indicates that the leaves are not necessary; their influence probably lies in keeping the stem better nourished.

On the other hand, the evidence seems to point to the second line of explanation, namely, that just as the growing shoots seem to exert a retarding influence on the buds below them, so the growing roots exert an influence which inhibits cells, otherwise able to do so, from forming roots. If, as just mentioned, the stem is cut off at its lower end and placed in water, and at the same time a portion of the stem higher up is surrounded with water, we get roots at both places. When these are growing well, if we cut off the lower part of the stem having the roots on and then submerge this end again, new roots soon appear at this point, although roots are growing vigorously a few centimeters above. In no case could I ever get a retarding influence of the roots to pass down the stem.

The problem to be solved in regeneration seems to be not so much the growth of parts following a removal (the causes here are those that induce growth everywhere), but the cause of non-development in the normal life of the plant; and this seems to lie in that influence which one part may exert over other parts or throughout the entire plant. A glance will show how universal this is among plants and the variety of ways in which it may manifest itself. If the main shoot of spruce is cut off, one or perhaps more of the dorsiventral plagiotropic lateral shoots will change their nature and become erect and radial. Many bulbous and tuberous plants do not produce seeds normally, but if the bulbs or tubers are removed seed production is then accomplished. This cannot be explained on the ground of specific bulb and seed forming substances, since if we assume the existence of these two substances, we must assume them to be different. GOEBEL (3, p. 213) says "in the normal condition the seed formation is hindered because the plastic material which might be used for the seeds streams into the bulb, where it is turned to account in the formation of bulbils for asexual reproduction." The assumption is that the nutritive materials, streaming to the point where the bulbs or tubers are to be formed, incite the formation of these organs; and if these are prevented from forming, the material will flow toward the flowers and there stimulate seed formation. This supposition is exactly the opposite of what actually occurs in plants. The nutritive, or any other soluble material, diffuses from its point of greatest density in all directions, as well toward the seed as toward the bulbs, and it will diffuse in one direction rather than the other because it is there being either changed or removed from solution by the activity of the cells. The "streaming" does not start the growth, but the growth activities remove the material from solution, and diffusion is set up in that direction due to the lowering of the concentration at that place. Growth or other cell activities involving a use or change of material must of necessity precede any movement other than diffusion in all directions. We have no reason for assuming that the food made in the leaves would diffuse toward the bulb any faster than toward the seed, and with the whole plant in a well-nourished condition it is scarcely possible that the amount available would be so slight that the embryos would still be in a condition of starvation so extreme that they could not even start to grow. If the latter were true, the diffusion of food materials from the leaves would be stronger toward this point than toward the tuber of any other point in the plant. There seems to be some factor dependent on the presence of growing tubers or bulbs which prevents the fertilized embryos from developing even in the presence of sufficient food and moisture. Kindred phenomena are common, *e. g.*, the death of the fern prothallium with the developing of the embryo. This cannot be due primarily to starvation, since each cell retains its own mechanism for food manufacture.

MORGAN (**11**, p. 272) has suggested that these phenomena are due to differences of tension existing throughout the plastic parts of the plants. "As long as the apical bud is present at the end of the stem or branch, or even near the apex, it exerts a pull or tension that holds the development of the parts in check; but if the apical bud is removed, the tension is relaxed and the chance for another bud developing is given." And further, MORGAN suggests that "from the apex of the plant to its base the tension is graded, being least at the apex and increasing as we pass to the base," so that, when the apex is removed, "those buds will develop first that are on the region of least tension, and then development will hold in check the other buds by increasing or establishing the tension on the lower part of the piece." Just what this "tension" may be is not very clear, and with GOEBEL I am unable to see that it makes the matter any plainer. MORGAN has suggested later (**12**) that if this idea of differences in tension is too vague, it can be given a more practical form by assuming it to be the outcome of osmotic differences in the cells. Diligent search has failed to reveal these in Salix or Phaseolus, and there seems to be no basis for the assumption.

MORGAN has more recently modified this hypothesis by another suggestion (**13**), according to which the difference in the development of buds at the two ends of a piece of stem is due to the relative state of development of the buds. In the willow, for example, those toward the apical end have reached a greater degree of maturity than those lower down, and so naturally are the first to develop. A few experiments will show this hypothesis to be quite untenable. Experiments already mentioned have indicated that when we take two pieces approximately alike, and remove all except the basal buds from one, these buds will develop simultaneously with the apical buds of the other. Indeed if any bud be selected, and the stem cut off just above it, the bud develops; but if the stem be cut off just below this same bud, so as to leave it at the base of the piece, it will not develop. Two pieces of Salix stem were selected as nearly alike as possible, and of exactly the same age. At about the center of each several buds, all alike, were selected; and each piece was cut in two, one so that these buds remained at the apical end, and the other so that they were at the basal end. In the former they developed and in the latter they did not. Experiments could be multiplied indefinitely to show that all the buds along the stem are equally able to develop; and whether any particular one does or not depends on whether the piece be cut so as to leave it near the apical or basal end.

The development of any plant involves the growth of a few and the suppression of many potential structures; and this is true not only of the vegetative buds, but also of other parts. In the ovary of Tilia, for example, ten ovules are present and may all be fertilized; but very soon nine cease activities and one only continues to form an embryo. A similar event occurs in Pinus and other plants. Were it merely a question of food, a fierce struggle would ensue among the developing embryos, and some at least would continue for a long time in a more or less starved condition. In Pinus practically all the embryos except one stop growing, while all about them are disintegrating tissues liberating food materials, some of which must pass by, or even through the arrested embryos to get to the one that continues growing. The formation of the embryo in many plants is accompanied—or immediately followed—by the development of other parts, often more or less distant, *e. g.*, the large fruit of the melon, an event that we cannot attribute to any increase of nutrition resulting from the developing of the embryo.

In addition to the growth at the meristematic growing points and along special regions which remain meristematic, as the cambium, the capacity for vegetative development is retained by many of the differentiated tissues in various parts of the plant body. That is, as the cells of the embryonic tissue differentiate into other forms and assume other functions they may still remain embryonic, in the sense of retaining complete reproductive capacity. So we find often matured cells of the leaf or cortex quite as able to form new organs as the cells of the meristematic apex of the shoot. In many cases, as *Tolmiea Menziesii*, *Cardamine pratensis*, *Asplenium bulbiferum*, *Camptosorus rhizophyllus*, in the ordinary course of development vegetative growing points arise on the leaves as well as on the shoot, and produce new members in the same way. As a rule, the more luxuriant the growth the more of these buds will be organized and develop; but usually, as in Tolmiea, even under the most favorable conditions not all the leaves on a plant will produce shoots. But I have found in this plant that every leaf produces a shoot when separated from the plant. In *Bryophyllum crenatum* there are numerous growing points along the margin of the leaf which do not usually develop further. They bear a similar relation to the growing points of the shoot as do the young axial buds of Salix to the growing point; for when the influence of these shoot buds is removed, those on the leaf form shoots.

On the other hand, in such leaves as Begonia the cells do not start to exercise this reproductive power by organizing growing points so long as they are in connection with other growing points of the plants; and GOEBEL showed that in Begonia, upon the removal of all the growing points of the stem, the leaf will organize them.

The same principle holds for other parts. In many roots the capacity for shoot development is expressed in the formation of "suckers," as in willows and other trees; but in other plants, probably the majority, as in Taraxacum, this ability seems able to express itself only when the influence of the shoot above has been removed.

Protoplasmic continuity from cell to cell throughout the entire living plant may fairly be accepted as demonstrated and the existence of various stimuli, either accelerating or retarding, emanating from different masses of tissue and affecting other even somewhat remote tissues is not at all impossible. Indeed, such a transmission of stimuli necessarily occurs in many of the tropisms, where the receptive region is separated by some distance from the region of response. The whole development of the plant body necessarily involves the suppression of many and the development of relatively few, either actual or potential, primordia; and the means by which this is accomplished (correlation if we must have a name) underlies in a most fundamental manner the entire organization of the plant. All the meristematic tissue and in many cases much of the differentiated tissue contains various potentialities of growth, potentialities which seem impossible of expression while in organic connection with certain growing parts. This interdependence of parts may be manifested in an inhibiting influence, as in the case of the roots or shoots mentioned, or in an accelerating effect, as in the growth of the fruit and adjoining parts after fertilization, or perhaps more correctly with the developing of the embryo. The experiments described indicate that the means of accomplishing this, that is the means by which, for example, a terminal bud suppresses the development of the other growing points on the stem or leaf, do not lie in the withdrawal by the former of the nutritive materials or the water. The theory of specific formative materials fails to account for it; nor does the tension hypothesis add anything to our knowledge of the process. Correlation, the endeavor of the plant to replace something lacking (DRIESCH), and form-stimulus (*Körperformreizen* of NOLL) are statements of the phenomenon and not at all explanations.

Protoplasmic stimuli emanating from various parts, reaching often throughout the entire organism, and affecting the behavior of the protoplasm of even remote portions are quite conceivable; so also are the formation and diffusion of ferments controlling growth; but we have yet no evidence of the existence of either.

SUMMARY.

A brief summary of the general conclusions thus far may be made as follows: The occurrence of regeneration in plants usually involves the replacement of parts removed, but the same result is often obtained when the organ is not removed, but is prevented from functioning. It is often inseparable from the ordinary growth of the plant, as for example when buds arise on the leaves of Tolmiea or Cardamine in ordinary course of the vegetative development of these plants; and the causes here are, no doubt, not different from those that induce the origin of buds on the growing points of the stem. The plant possesses innumerable growing points either organized or potential, the vast majority of which must not be allowed to develop if the plant body is to retain anything like a definite organization. In most cases this development does not occur in the ordinary life of the plant, because these cells, capable of producing new organs, are held in check by those parts already growing. This non-development does not seem to be due to any lack of those conditions that favor growth, as nutrition and moisture; or to such influences as light and gravity; or to a lack of definite "formative substance;" but to some influence independent of all these, which an organ, acting perhaps along the protoplasmic connections, is able to exert over other parts and so prevent their growth. When this influence is removed, the favorable growth condition, present all the time, permits the growth of the part to occur. In such a controlling influence of growing organs over the numerous potential growing points throughout the plant there exists very evidently a principle of fundamental importance in plant organization.

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